Use of Diatoms as Markers of Sea Turtle Movements in Martinique: Initial Results, Prospects and Potential

Utilización de Diatomas como Marcadores de Movimientos de Tortugas Marinas en Martinica: Resultados Iniciales, Perspectivas y Potencial

Utilisation des Diatomées comme Marqueurs des Déplacements des Tortues Marines en Martinique: Premiers Résultats, Perspectives et Potentiel

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ABSTRACT

The 7 sea turtle species are found in all the seas of the world. Some of them have a clear predilection for the equatorial zones where they must come to lay eggs. The hawksbill turtle is one of the main species to lay eggs on the beaches of Martinique. The present study explores and compares the diversity of diatoms communities growing on the shell of nesting hawksbill turtle sampling in 3 beaches in Martinique (West Indies Island) using light microscopy. This is the first study on the global diatom communities (counting and observations) on this turtle species.

The exploratory analysis on the counting effort to standardize the counting process for all turtle species demonstrated that diatom richness is largely underestimated. Hawksbill turtle are great reservoirs of diverse genera and diatom species many still unknown, probably new and require substantial taxonomic work.

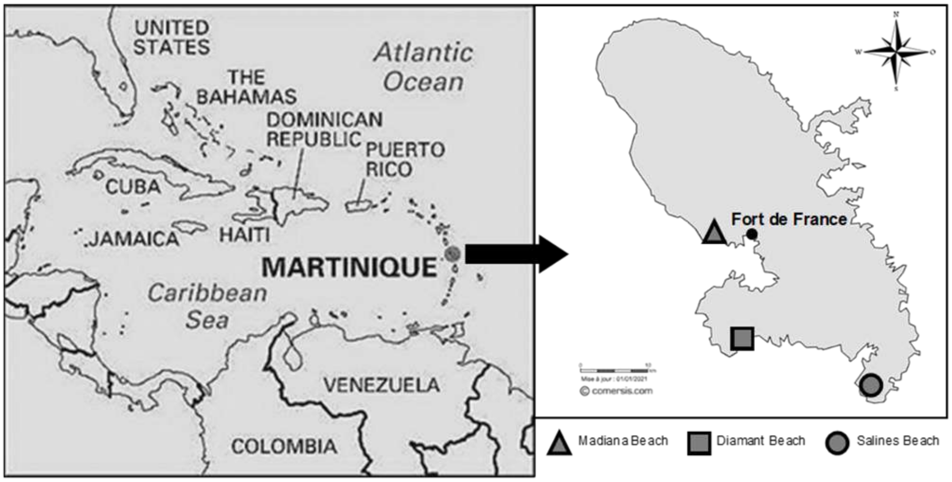
There is poor correlation between turtle diatom communities and no correlation with the sampling beach. The community variations depend on individuals. The diatom turtle communities are often benthic diatom coming from the turtle environment and are related to the turtle behavior. A comparative analysis of diatom local flora (sea grass bed and coral reef), world marine flora, and turtle communities could allow a better understanding of turtle habitats and behaviors at local scale, but also largely scale. Learning about other organisms living with turtles, environmental contaminations of their habitats by experimental way of using eDNA data of biofilm and the adsorption capacity of EPS have yet to be performed.

INTRODUCTION

A better understanding from sea turtle migrations, thanks to the microflora that develops on their shells, will help us to a better understanding of their behavioral patterns, physiological processes and migratory routes (Rivera et al., 2018). This is an essential part of the challenge: to be able to implement global management measures that will allow these species to be protected on a large scale.

This non-invasive, protocol is therefore highly relevant and potentially rich in information on turtle ecology and movements. It employs a taxonomic approach using microscopy and molecular analysis, focusing on the bio-regionalism, diversity and ecology of diatom assemblages. Actually, there are several taxonomic publications on epizoic turtle diatoms (Majewska et al., 2017, 2015a, 2017a; Riaux-Gobin et al., 2019, 2017a, 2017b), and six major references on the relationship between the diatom community composition and the turtle behavior and movements. The first studies on epizoic diatoms were carried out on whales at the beginning of the 20th century (Bennett et al., 1920). Then first studies carried out on reptiles (in this case turtles) were first focused on the macrobiota (Kitsos et al., 2005; Pfaller et al., 2008a, 2008b) and then, very recently on the microbiota (Majewska et al., 2015a, 2015a; Robinson et al., 2016).

Robinson et al. (2016) demonstrated for the first time the presence of diatoms on the shells from the seven existing sea turtle species. This study showed that each host species carries at least two species of diatoms for a total of 18 species observed (using scanning microscopy). It also calls for further research to determine whether these species are strict or optional commensal diatoms, and whether micro-epibiont species are associated with their host species. The observed diatom assemblages are partly dependent on the host species. Although not exclusive, some taxa are particularly associated with each turtle species. Riaux-Gobin et al., (2021) studying four different sea turtle species, noticed that Hawksbill turtles, *Eretmochelys imbricata*, hosted the most diverse diatom communities from other species. This diversity is probably due to



FiguFigure 1. Map showing the location of the study area and the 3 beaches (Madiana Beach, Diamant Beach and Salines Beach).

their feeding area in shallow littoral zones.

Assemblages also depend on the sites frequented by the turtles. Riaux-Gobin et al (2021) have shown on a large number of samples (124 individuals studied, 4 turtle species, 3 geographical areas) that certain commensal diatom species identified on the shells on Green turtles, *Chelonia mydas*, from Caribbean are completely absent from the shells of Green turtles from Indo-Pacific. Van de Vijver et al. (2020) studied Loggerhead turtle, *Caretta caretta*, individuals from Croatia, Greece, South Africa and Florida and confirmed the bio-regionalism of epibiontic diatom assemblages.

The differences observed between turtle assemblages living in different sites may be linked to biogeography and/ or environmental conditions. A study carried out on turtles living in the Caribbean Sea in Costa Rica (CR) and in the Persian Gulf in Iran (GPI) (Majewska et al., 2017b) showed that the assemblages observed in Costa Rica were much more abundant and diverse in terms of species and life form than those in Iran (22 CR species vs. 12 GPI). These differences can be attributed to the more constraining natural and anthropogenic conditions in the Persian Gulf, which offer a lower diversity of turtle feeding sites.

The studies of assemblages can also help to distinguish the physiological stages of individuals and/or their behavior. For example, the more diversified assemblages of juveniles made it possible to distinguish them from adults of the same species living in the same study area (Riaux-Gobin et al., 2021). This result can be explained by the difference in feeding behavior according to physiological stage.

The study of Loggerhead Turtle samples from Croatia, Greece, South Africa and Florida has permitted to distinguish 400 diatom species from 100 different genera. Among the identified taxa, the contribution of strictly epizoic diatoms is relatively minor compared to benthic diatoms, which is explained by the species' feeding behavior (Van de Vijver et al., 2020). The authors conclude that Loggerhead turtles are reservoirs and probably vectors of benthic diatoms, even if their role in the dispersal of benthic diatoms is still poorly understood. Other studies confirm that the majority of commensal diatoms are benthic and not strictly epizoic. In the study of Rivera et al. (2018) only 2 species are considered strictly epizoic (with no certainty for *Labellicula lecohuiana*), the others being benthic and originating from seagrass beds.

This result is crucial, as it justifies the relevance of studying commensal diatoms for understanding their host behavior and movements (Majewska et al., 2017b). The present study was conducted on the commensal diatom communities of nesting Hawksbill turtles from various beaches in Martinique, with objective to provide data on their diversity and species composition and their possible relationships with nesting beaches and local flora. Additionally, the method used to estimate the diatom biodiversity and community structure was explored in order to developed a standardized method for counting diatom samples of turtle shells.

MATERIAL AND METHODS

Study Area

Data were collected from three beaches (Madiana, Diamant and Salines) of Martinique, French West Indies Island (Figure 1). Madiana Beach is located near Fort-de-France in the central zone on the Caribbean Sea side, Diamant Beach in the south on the Caribbean Sea side, and Salines Beach on the south Atlantic sea side.

Diatom Sampling Protocols

Biofilm was collected on nesting turtles using a toothbrush which was rinsed in Falcon tubes pre-filled with ethanol (96%). The operation was reproduced in several place on the shell (Wu, 2016) using standardized diatom sampling protocol for nesting sea turtles outlined by Pinou et al. (2019). Toothbrush was systematically replaced for each turtle sampled.

Each sample has a referencing number: Date (year/ month/day)-Beach (M, D or S)-Turtle species (EI for

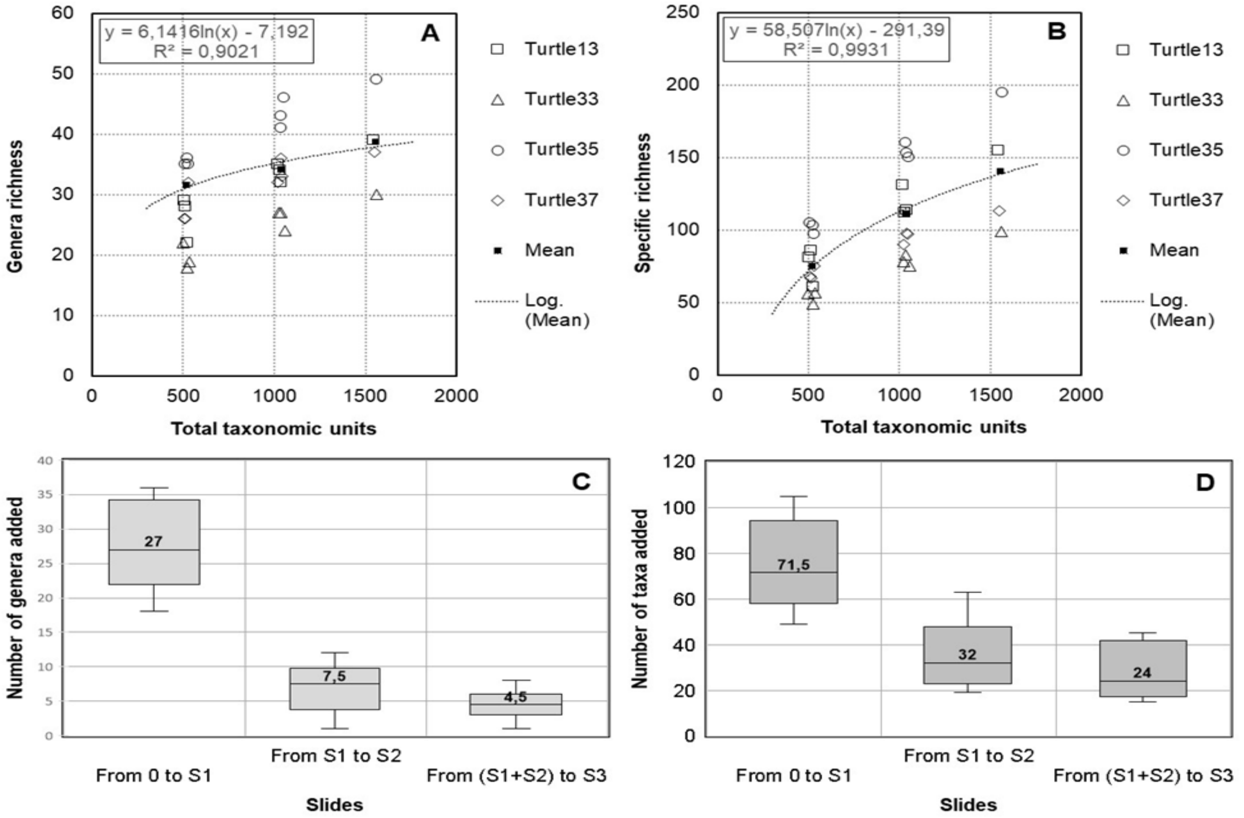


Figure 2. Accumulation curve for each turtle depending on counting effort (A. Genera, B. Species. Mean was calculated with all "1 slide" counting, all "2 slides" counting and all "3 slides" counting with an extrapolation logarithmic curve) and box and whisker plot of taxa unit gain by adding slide counting (C. Genera, D. Taxa. Whiskers indicate maximum and minimum. Median value is denoted within the box.).

Hawksbill Turtle)-Specimen Number and each turtle have a global reference number to facilitate the analyses. Thirty-eight samples were collected on Hawksbill turtles in July and August 2020. The list of the 10 samples processed in this initial study is given in Table1.

The samples were stored in a fridge at 4°C. One part of the sample was used to prepare 3 permanent slides using Naphrax mounting medium for diatom observations in optical microscopy (CEN. EN 13946, 2024) and counting (CEN. EN 14407, 2014). The remaining portion was stored for Scanning Electron Microscopy and possibility of DNA analysis.

Counting Protocole

Permanent slides were analyzed using an Olympus BX53 microscope equipped with differential interference contrast (Nomarski) optics and the Olympus UC90 Imaging System.

In each slide, a minimum of 500 diatom valves (between 500 and 544) were counted and identified in random transects to estimate the species richness and composition in the samples. After counting, the complete slide was examined to record all occurring taxa in a sample. Extensive literature including books and other taxonomic publications was used to identify the observed taxa listed in Supplement Table 1.

The taxa recorded in the 10 samples are listed in the supplementary Table 2.

All the taxa observed were photographed, compiled into identification plates to produce a turtle diatom floristic atlas with unidentified taxa numbered to allow a standardized counting of species. Diatoms are classified into major morphological groups: Centric, Araphid, Monoraphid, Eunotioid, Asymmetric Biraphid, Symmetric Biraphid, Nitzschioid; Epithemioid and Surirelloid. Unidentified genera (identification criteria invisible under light microscopy or unknown) are pooled into these morphological groups but taxa unit have a unique numbering.

Due to the lack of standardized method (Table 2), an exploratory analysis to determine the number of taxonomic units needed to assess diatom biodiversity with light microscopy was a necessary requirement before proceeding with data analysis (Table 2).

Data analysis

The floristic list of 3 slides were mixed and permutate for each turtle (slide 1, slide 2, slide 3, slides 1+2,

Table 1. Listing of the 10 samples selected for counting in this study and the 4 samples randomly selected for the exploratory analysis (*).

Date	Beach	Reference Code	Turtle number	Slides Number
2020-08-19	Diamant	20200820-D-EI-01	1	1
2020-07-13		20200713-D-EI-01	37 *	1, 2 and 3
2020-08-01	Madiana	20200801-M-EI-01	6	1
2020-07-20		20200720-M-EI-01	13 *	1, 2 and 3
2020-07-11		20200711-M-EI-02	35 *	1, 2 and 3
2020-08-04	Salines	20200804-S-EI-01	4	1
2020-07-17		20200717-S-EI-01	8	1
2020-07-13		20200713-S-EI-03	9	1
2020-07-19		20200719-S-EI-02	11	1
2020-07-13		20200713-S-EI-02	33 *	1, 2 and 3

slides 1+3, slides 2+3, slides 1+2+3) to obtain a matrix for an accumulation curve of taxonomic richness as a function of the number of taxonomic units counted. The mean was calculated for 1 slide counting, 2 slides counting and 3 slides counting with a logarithmic curve.

To make pair-wise comparison between the turtle-slide couple for genera and taxa we used the Pearson method (Pearson, 1895) with the Corrplot package (Wei and Simko, 2024). To make pair wise comparison between turtles for genera and taxa, we used the Spearman method (Spearman, 1904) with Corrplot package. The CFA analysis was conducted with ade4 (Dufour, 2007), factoextra (Kassambara and Mundt, 2020), FactoMineR (Le et al., 2008) and ggplot2 (Wickham, 2016) package. The Pearson's Chi-test was performed with Stats (R Core Team, 2024) with Corrplot package.

RESULTS

Exploratory analysis

The accumulation curve (Figure 2) indicated a very good correlation between genera richness (Figure 2A) and taxonomic richness (Figure 2B) across an increasing range of counting effort. The results also showed that the real biodiversity of the samples is not reach because there is no

Table 2. Listing of counting methods in bibliographic studies. G = Green Turtle, H = Hawksbill Turtle, KR = Kemp'sRidley Turtle, L = Loggerhead Turtle, OR = Olive Ridley Turtle. LM = Light Microscopy, SEM = Scanning Electron Microscopy.

Bibliographic Data	Turtle Species	Microscopic Methods	Counting Methods	Total TU Observed
Majeska et al. (2015b)	OR	LM	Surface area of 2mm²	Unknown
Robinson et al (2016)	G, H, KR, L, OR	SEM	Presence/absence	Unknown
Majeska et al. (2017b)	G	LM	Surface area of 2mm²	Unknown
Rivera et al. (2018)	G	LM	European standard EN 14407	400 valves
Kaleli et al. (2020)	L	LM	Unknown	Unknown
Van de Vijver et al. (2020)	L	LM	European standard EN 14407	400 valves
		LM	Complete slide	All Occurrent Taxa
Riaux-Gobin et al. (2021)	G, H, OR, L	SEM	Presence/absence	Unknown
Kaleli et al. (2023)	L	LM	Unknown	300 valves

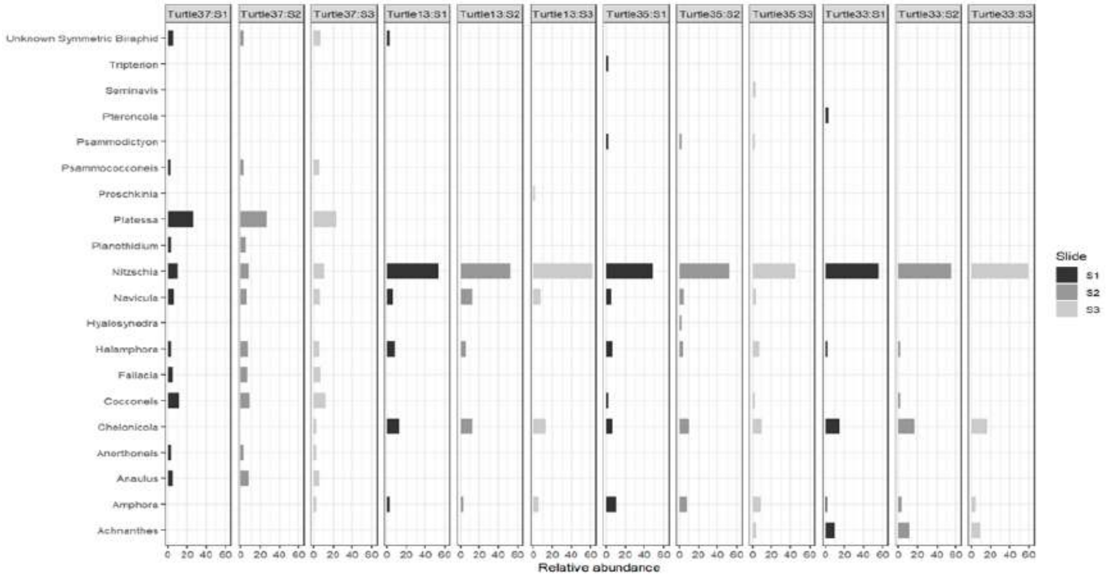


Figure 3. Relative abundance of genera for each turtle-slide couple. Only most contributive genera shown on the graphic (cumulative genera abundance ≥ 2%). Unknown Symmetric Biraphid = unidentified genus belonging to the Symmetric Biraphid morphological group. S1=Slide1, S2=Slide 2 and S3=Slide 3.

asymptote. The genera (Figure 2C) and species (Figure 2D) discovery slow down but remain high, especially for species.

The most abundant genera are *Platessa* for Turtle 37 (Diamant Beach), *Nitzschia* for turtles 13, 35 (Madiana Beach) and 33 (Salines Beach). The most contributive genera appear in the first counting (S1) and remain constant in the 3 counts (Figure 3). The genera whose appear in only one or 2 slides remain.

The same pattern is observed for the most contributing taxa (Figure 4), but is more difficult to observe for the secondary taxa. Both genera and taxa, from the same turtle are strongly correlated (Figure 5). Turtles 13, 33 and 35 are strongly correlated (Figure 5A), probably due to the dominance of the genus *Nitzschia* in the 3 communities. Turtle 37 is the most lightly correlated with the other turtles.

The taxon-based correlogram shows differences and poorer correlation between species communities. Even if genera are common, the species within are different between turtles, with specificity for each individual regardless of slide.

Richness and species composition for the 10 turtles

A total of 555 taxa (including species, varieties and forms) were identified during the counts. Among these taxa, 48 have not been assigned to a genus and represents at least 25 different taxonomic groups (which could be additional genera). The other taxa belong to 77 identified genera.

Outside the count procedures, 896 additional taxa were observed bringing the total number of recorded taxa to 1451, belonging to 131 identified genera and 41 groups of unknown taxa. Only 17% (243 taxa) of all taxa could be identified to the species level, 72% (1046 taxa) on the genus level and 11% are just identified to the morphological group (162 taxa).

The most taxon-rich genera found in all samples included *Nitzschia* (173 taxa), *Navicula* (168 taxa), *Cocconeis* (91 taxa) and *Halamphora* (77 taxa), *Amphora* (66 taxa), *Mastogloia* (63 taxa), *Licmophora* (41 taxa), *Diploneis* (36 taxa) and *Fallacia* (33 taxa). (Table 3). Diatom genus composition differed among samples from different beaches. The total flora on hawksbill turtle sampled in Madiana and Salines beaches contained mostly *Nitzschia* (117 and 87 taxa) and *Navicula* (102 and 70 taxa) whereas in Diamant samples these two genera are less diversified: *Navicula* (46 taxa) and *Nitzschia* (42 taxa).

The counting flora on hawksbill turtle sampled in Madiana and Salines beaches also contained mostly *Nitzschia* (148 and 94 taxa) and *Navicula* (56 and 26 taxa) associated with *Halamphora* (48 and 31 taxa). The counting flora of Diamant samples are characterized by *Navicula* (27 taxa), *Cocconeis* (21 taxa), *Halamphora* (21 taxa), *Nitschia* (20 taxa) and *Fallacia* (19 taxa).

Madiana samples show the most diversified flora both for presence and counting (915 and 596 taxa), whereas, Diamant samples the lowest specific richness (456 and 264 taxa). A comparison between the 10 hawksbill samples and literature data is illustrated in Figure 6 and shows that richness observed on hawksbills turtles is greater than on other species. Several common diatoms found on carapace of hawksbill turtles are illustrated in Figure 7.

significant variations in spotted eagle ray sightings across

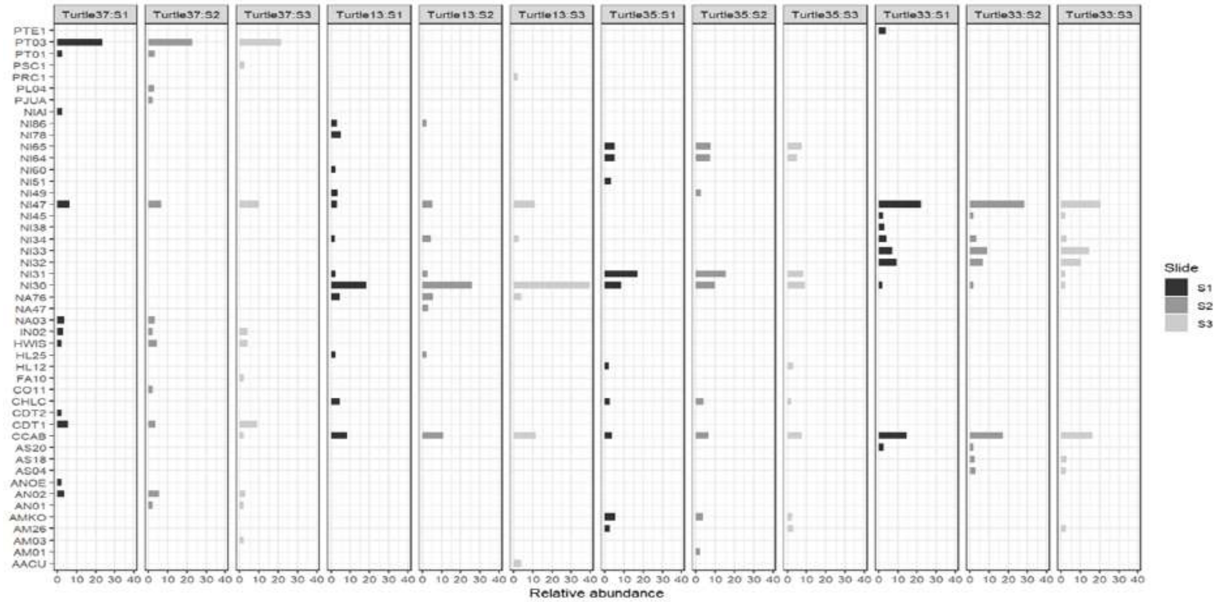


Figure 4. Relative abundance of taxa for each turtle-slide couple. Only most contributive taxa shown on the graphic (taxa abundance ≥ 2%). S1=Slide1, S2=Slide 2 and S3=Slide 3. Taxa and correspondence between taxa code and species name listed supplement Table 2.

the different islands of FWI (Figure 2). Sightings are most frequent in Saint-Barthélemy, where the species is classified as "common", with a presence encounter rate of 32%. In contrast, encounter rates are significantly lower in Guadeloupe (3%) and Martinique (1%), where the species is considered as "rare". In Saint-Martin, the spotted eagle ray is classified as an "occasional species", with an encounter rate of 13%.

The infrageneric similarity index showed that some turtles diatoms communities are related and share a similar genera community (Spearman values > 0,5) but not related with the beach sampling (Figure 8A). The interspecific similarity index indicated a poor relation between samples and no relationship between diatom communities sampled on the same beach (Figure 8B). The CFA, based on genera, highlights 2 turtles that stand out from the rest (Figure 9).

Turtle 6 (Madiana beach) is characterized and strongly associated with *Hyalosira* and *Neosynedra*, and Turtle 37 (Diamant beach) with *Platessa* with more slightly association with *Anaulus*, *Anorthoneis*, *Cocconeis*, *Fallacia*, *Planothidium*, *Psammococconeis* and *unknown Symmetric Biraphid* (Figure 11).

There is a strong opposition between Turtle 37 and the others on the F1 axis, and between Turtle 6 the others on F2 axis. Both are negatively associated with *Chelonicola* and *Nitzschia* (Figure 9). A second CFA was conducted without these two turtles (Figure 10) because of weight of Turtles 6 and 37 on the CA biplot with genera.

Turtle 1 (Diamant beach) is positively associated with *Hyalosira* and *Halamphora* but negatively associated with *Nitzschia* (Figure 9 and Figure 11). Turtles 13 and 35 (Madiana beach), Turtle 33 and 8 (Salines beach) are positively associated with *Nitzschia*. Turtle 35 is also positively associated with *Psammodictyon* and *Tripterion*, Turtle 33 more strongly with *Achnanthes* (Figure 9 and Figure 11). Turtle 11 is strongly associated with *Chelonicola*, while Turtle 4, with *Chelonicola*, and *Licmophora* and *Pteroncola*. Both are negatively associated with *Nitzschia* (Figure 9 and Figure 11).

Turtle 9 is strongly associated with *Proschkinia*, and slightly with *Halamphora*, *Hyalosynedra* and *Mastoglioa*, and negatively related to *Chelonicola* and *Hyalosira*. (Figure 9 and Figure 11). As same as genera, CFA, based on taxa, highlights 2 turtles that stand out from the rest (Figure 12).

Turtle 6 is associated with *Hyalosira parietina* Witkowski Lange-Bertalot & Metzeltin*, Neosynedra provincialis* (Grunow) Williams et Round*, Hyalosira* sp9 and *Amphora acutiuscula* Kützing, and Turtle 37 (Diamant beach) strongly with *Platessa* sp3 and *Cocconeis* cf *distantula*1 (Figure 12 and Figure 14).

There is a strong opposition between Turtle 37 and the others on the F1 axis, and between Turtle 6 and the others on F2 axis. Both are negatively associated with *Chelonicola caribeana* Riaux-Gobin, Witkowski, Kociolek (Figure 12).

Turtle 6 is associated with *Hyalosira parietina* Witkowski Lange-Bertalot & Metzeltin*, Neosynedra provincialis* (Grunow) Williams et Round*, Hyalosira* sp9 and *Amphora acutiuscula* Kützing, and Turtle 37 (Diamant

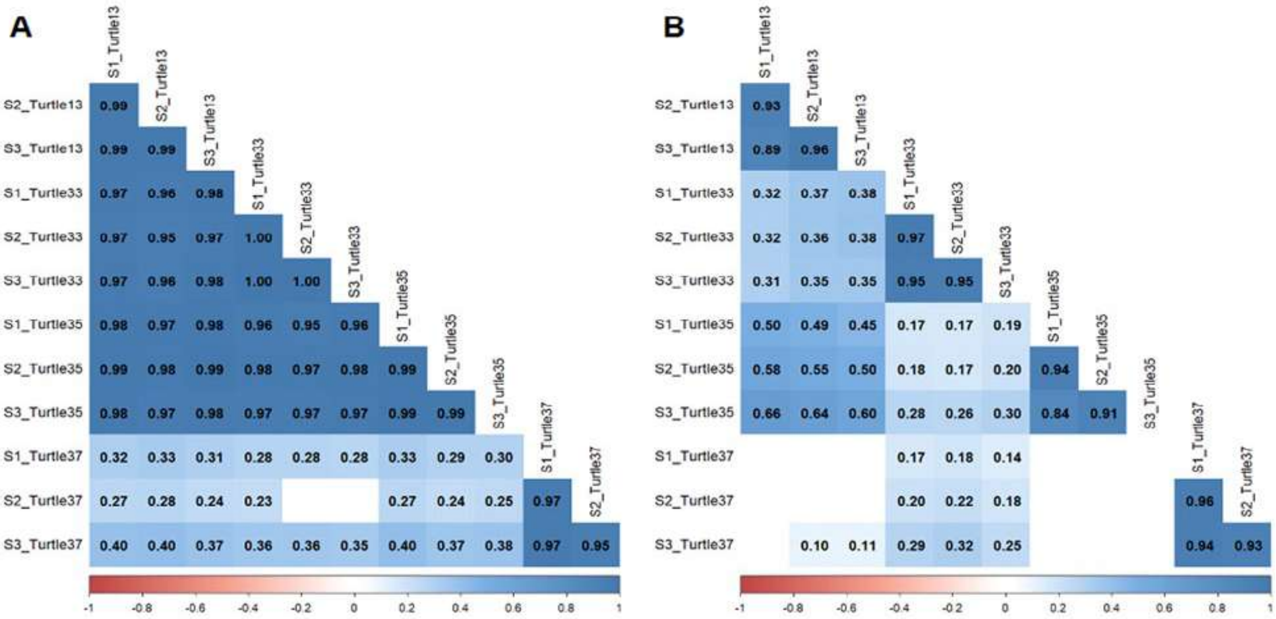


Figure 5. Correlogram of Pearson index between Turtle-Slide couple for Genera (A) and Taxa (B). Matrix of p values used to applicate a significative rate of 0,05 to eliminate non significative correlation coefficient (blank). Diagonal has been removed. S1=Slide1, S2=Slide 2 and S3=Slide 3. Coefficient value is denoted in each square.

beach) strongly with *Platessa* sp3 and *Cocconeis* cf *distantula*1 (Figure 12 and Figure 14).

There is a strong opposition between Turtle 37 and the others on the F1 axis, and between Turtle 6 and the others on F2 axis. Both are negatively associated with *Chelonicola caribeana* Riaux-Gobin, Witkowski, Kociolek (Figure 12).

A second CFA was conducted without these two turtles (Figure 13) because of weight of Turtles 6 and 37 on the CA biplot with taxa. Turtle 1 is positively associated with *Halamphora* sp63, *Hyalosira* sp18 and *Hyalosira hesperia* Álvarez-Blanco & S.Blanco (Figure 13 and Figure 14). Turtles 13 is strongly associated with *Nitzschia* sp30 (Figure 13 and Figure 14).

Turtle 35 is strongly associated with *Nitzschia* sp31, slightly with *Amphora kolbei* Aleem and *Nitzschia* sp30 but negatively with *Chelonicola caribeana* (Figure 13 and Figure 14).

Turtle 33 is associated with *Nitzschia* sp47, and slightly with *Nitzschia* sp32 and *Nitzschia* sp33 (Figure 13 and Figure 14). Turtle 8 is positively associated with *Nitzschia* spp 38, 33,32 and 45 (Figure 13 and Figure 14).

Turtle 11 is strongly associated with *Chelonicola caribeana*, while Turtle 4, with *Chelonicola caribeana*, *Halamphora* spp 29 et 62 and *Pteroncola* sp1. Both are negatively associated with *Nitzschia* species (Figure 13 and Figure 14).

Turtle 9 is associated with *Proschkinia* sp1, and slightly with *Halamphora* sp9, *Halamphora* cf *tenerrima* 3 and *Nitzschia* sp45, and negatively related to *Chelonicola caribeana* (Figure 13 and Figure 14).

DISCUSSION

Exploratory analysis

Currently no studies have employed light microscopy and counting to examine the presence of diatoms in Hawksbill turtles. Instead, studies have relied on the observation of the presence/absence of diatoms, with only partial observations (Robinson et al., 2016; Riaux-Gobin et al., 2021. For the other turtle species, the method used to count diatoms differ among authors. Thus, investigations on the counting effort were necessary to obtain a standardized method which can be applied on all samples and to allow (i) interspecific turtle studies, (ii) biogeographic studies and (iii) year to year comparison for same individuals.

Our investigations on 4 hawksbills turtles indicate that genera and especially species richness of these diatom communities are largely underestimated by counting 500 valves on only one slide, and are not reached with a total of 1500 valves on 3 slides. More research is needed on other turtles to decide how many total taxonomic units are needed to better understand the biodiversity of the diatom communities attached to the shells of Hawksbill turtles.

Even if the true taxonomic richness is unknown, the community structure shows unique specificities from the first slide for the 4 turtles tested and allowed comparative analysis on the 10 samples.

Richness and species composition for the 10 turtles

The observed taxa richness is clearly higher than currently observed from any other sea turtle species

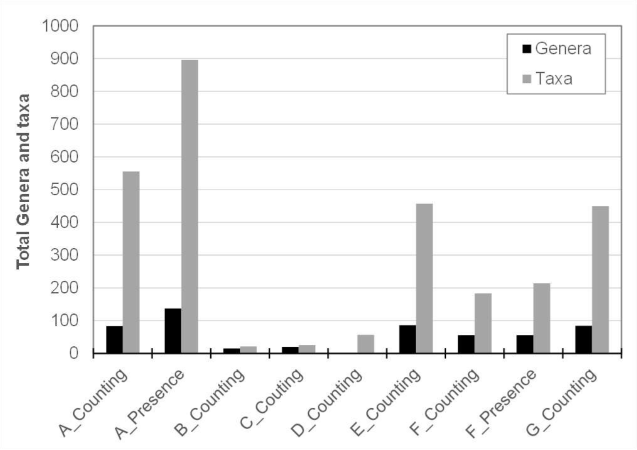


Figure 6. Comparison of genera and taxa richness with scientific literature data. A = our study on 10 Hawksbill, B= Majewska & al (2015b) on 55 Olive Ridley, C = Majewska & al (2017b) on 125 Green, D = Rivera & al (2018) on 7 Green, E = Kaleli & al (2020) on 33 Loggerhead, F = Van de Vijver & al (2020) on 13 Loggerhead, G = Kaleli & al (2023) on 200 Loggerhead.

sampled so far. As all the observed taxa were numbered, some authors, such as Van de Vijver et al. (2020), grouped several unidentified taxa, mostly belonging to *Amphora*, *Navicula* and *Nitzschia* under common name. This approach certaintly reduced the number of taxa and underestimate the real richness of their samples. It is also possible that the applied sampling technique, namely the use of razor blade or scalpel on a limited surface of the carapace, (Majewska et al., 2015b, 2017b; Riaux-Gobin et al., 2021) in contrast with the application of a toothbrush for brushing on a larger surface may have exerted an influence on the observed taxon richness. The results also indicated an influence of the sampling technique, as reported by Van de Vijver et al. (2020). Brushing the surface with a hard toothbrush removes more efficiently the well-attached, adnate diatom taxa from the hard carapaces and it was designated as the standard sampling method for epizoic diatom communities (Pinou et al., 2019).

Only 155 of the 1451 species observed have already been recorded in Martinique and only 157 have been recorded in Caribbean Sea (Supplement Table 1). SEM and molecular analysis would be required to clarify the correct identity of certain numbered taxa, but there are also a large number of new taxa or even genera to describe.

Turtle diatom communities sampled on the same beach share the most frequent genera but few species. The most abundant genera are mainly common to the 10 turtles studied but shows significative differences between turtles. These differences are not correlated to the sampling beach. The correlation between turtles at the species level is very poor and no correlation between genera and the sampling beach.

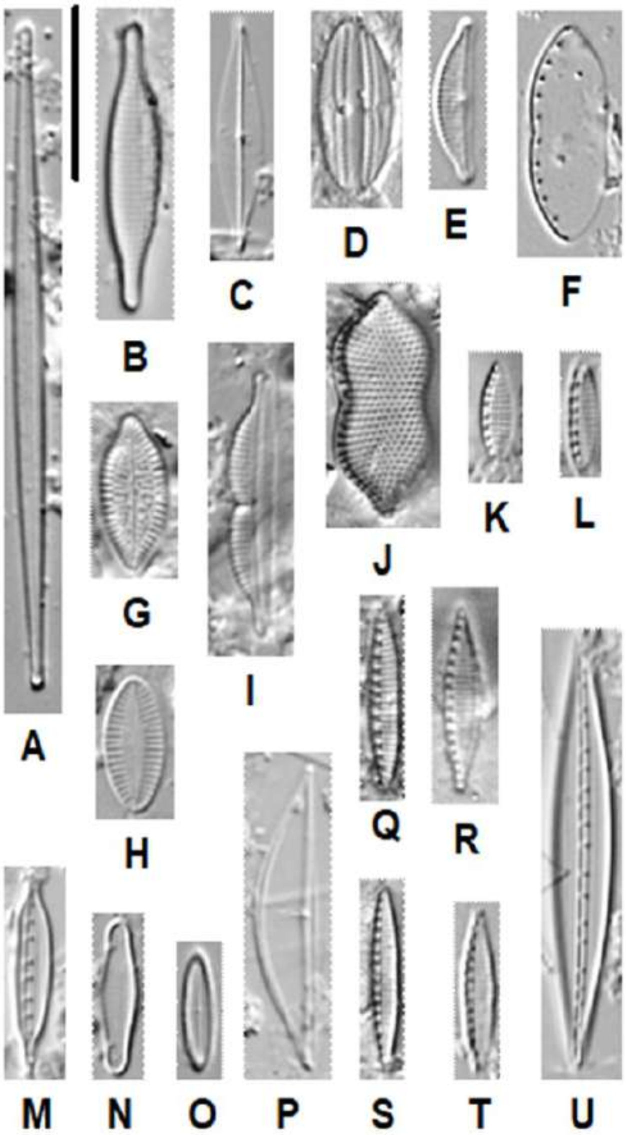
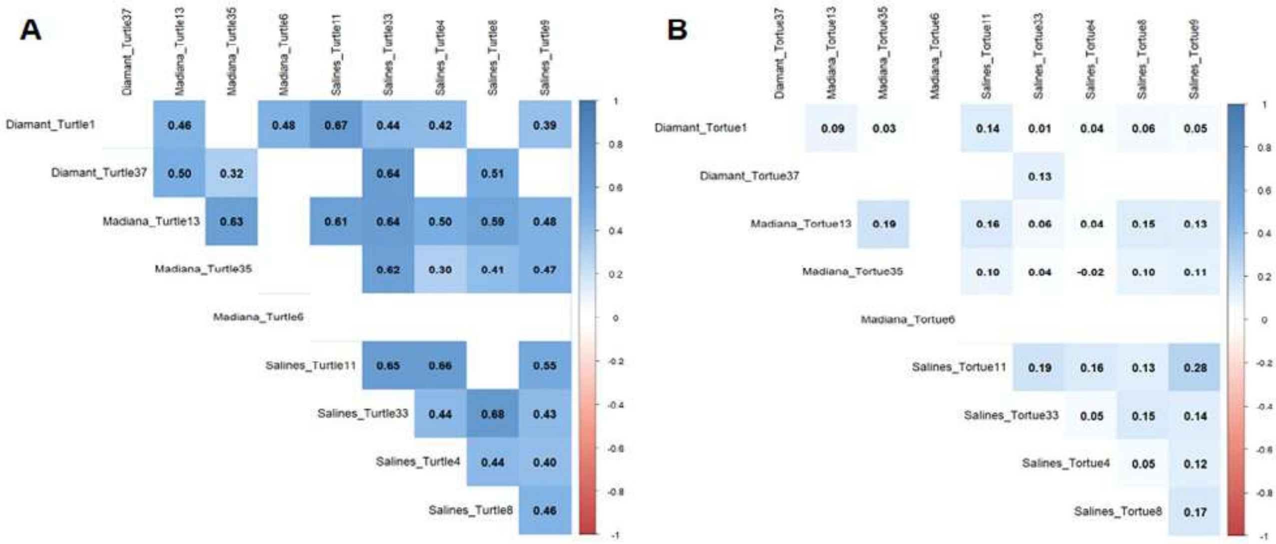


Figure 7. Light microscopy photographs of diatom taxa associated with hawksbill sea turtles. A: Hyalosynedra sp1, Turtle 13; B: Hyalosira sp18, Turtle 1; C: Proschkinia sp1, Turtle 13; D: Amphora exilitata Giffen, Turtle 33; E: Halamphora sp9, Turtle 9; F: Nitzschia amabilis H. Suzuki, Turtle 35; G: Platessa sp1, Turtle 37; H : Platessa sp3, Turtle 35; I: Halamphora kolbei (Aleem) Álvarez-Blanco & S.Blanco, Turtle 35; J: Psammodictyon panduriforme var. continua (Grunow) Snoeijis, Turtle 33; K: Nitzschia sp31, Turtle 13, L: Nitzschia sp30, Turtle 35; M: Nitzschia volvendirostrata Ashworth, Dabek & Witkowski, Turtle 13; N: Hyalosira parietina Witkowski, Lange-Bertalot & Metzeltin, Turtle 6; O: Chelonicola caribeana Riaux-Gobin, Witkowski, Ector & D.Chevallier, Turtle 11; P: Halamphora sp63, Turtle 1; Q: Nitzschia sp47, Turtle 33; R: Nitzschia sp 33, Turtle 8; S: Nitzschia sp45, Turtle 33; T: Nitzschia sp 32, Turtle 4; U: Nitzschia angularis var. affinis (Grunow) Grunow, Turtle 1.Scale bar represent 10 µm.



	Diamant_Turtle37	Madiana_Turtle13	Madiana_Turtle35	Madiana_Turtle6	Salines_Turtle11	Salines_Turtle33	Salines_Turtle4	Salines_Turtle8	Salines_Turtle9
Diamant_Turtle1		0.46		0.48	0.67	0.44	0.42		0.39
Diamant_Turtle37		0.50	0.32			0.64		0.51	
Madiana_Turtle13			0.63		0.61	0.64	0.50	0.59	0.48
Madiana_Turtle35						0.62	0.30	0.41	0.47
Madiana_Turtle6									
Salines_Turtle11						0.65	0.66		0.55
Salines_Turtle33							0.44	0.68	0.43
Salines_Turtle4								0.44	0.40
Salines_Turtle8									0.46

	Diamant_Tortue37	Madiana_Tortue13	Madiana_Tortue35	Madiana_Tortue6	Salines_Tortue11	Salines_Tortue33	Salines_Tortue4	Salines_Tortue8	Salines_Tortue9
Diamant_Tortue1		0.09	0.03		0.14	0.01	0.04	0.06	0.05
Diamant_Tortue37						0.13			
Madiana_Tortue13			0.19		0.16	0.06	0.04	0.15	0.13
Madiana_Tortue35					0.10	0.04	-0.02	0.10	0.11
Madiana_Tortue6									
Salines_Tortue11						0.19	0.16	0.13	0.28
Salines_Tortue33							0.05	0.15	0.14
Salines_Tortue4								0.05	0.12
Salines_Tortue8									0.17

Figure 8. Correlogram of Spearman index between Turtles for Genera (A) and Taxa (B). Matrix of p values used to applicate a significative rate of 0,05 to eliminate non significative correlation coefficient (blank). Diagonal has been removed. Coefficient value is denoted in each square.

Commensal diatoms are often benthic diatoms coming from the environment during the migration and feeding areas, with little exchange between turtles. The truly epizoic diatoms are studied by several taxonomists but not well known for sure and the percentage of these taxa in the diatom communities is probably low (Van de Vijver et al., 2020). Given their rate of multiplication, benthic diatoms communities are considered to reflect integrated environmental conditions from 1 to 2 or even 3 months prior to sampling. In an oral presentation at the ADLaF 2024 symposium, Margaud Pottiez (Junior Scientist of Meise Botanic Garden, Nieuwelaan 38, 1860 Meise, Belgium) showed that community changes occur after one month in a new environment (unpublished data). These experiments have been carried out in the recovery ponds for injured Loggerhead turtle. The diatom community was very different when the arrived turtles and became more similar to the environment diatom community after 1 month. The diatom communities of turtles 6 and 37 are very different than others and the 2 turtles differs significantly in genera and species composition. These 2 communities are negatively associated with *Chelonicola caribeana* Riaux-Gobin, Witkowski, Kociolek, a species which is dominant and relatively abundant on Martinique turtles (Riaux-Gobin et al., 2017b). It is likely that these turtles have just arrived in Martinique. Recent studies have shown that Hawksbills turtles are also great travelers and not necessarily sedentary as previously thought. And because the communities vary greatly, probably come from the different places.

The other 8 turtle communities exhibit greater similarity, with the composition of genera and taxa varying depending on the individual. Generic and specific community differences probably indicate different feeding areas. Due to the specificity of each diatom community, a comparative analysis of the turtle and seagrass diatom communities would allow the identification of the different feeding areas according to individuals. Further investigations on local flora, of seagrass beds and coral reefs, and, in more large scale, in Caribbean Sea would provide precious information on the turtle environment and on the diatom exchange between those from turtles and marine diatoms.

Perspectives

Diatom communities indicate that biofilms on turtles are different. The biofilm taken from turtle shells could be analyzed in different ways in addition to the morphological study of benthic diatoms. Biofilm is an aggregate of microorganisms composed of algae, fungi, bacteria, protozoa, metazoans, and other non-biological materials, in which cells are integrated into extracellular polymeric substances (EPS) produced by themselves (Flemming et al., 2016). These EPS are mainly composed of polysaccharides, proteins, lipids and extracellular DNA (eDNA) (Flemming and Wingender, 2010). Biofilms are complex absorbent systems using different binding mechanisms and sites in the cytoplasms of biofilm cells, at the cell walls of biofilm cells and at the EPS level. These binding sites include anionic and cationic exchangers, implying that many different substances can be trapped and accumulated by biofilm cells, even when such compounds are present at very low concentrations. The adsorption and absorption capacities of biofilm concern several types of substances such as nutrients, metals or various toxic substances (lipophilic and even hydrophilic) (Flemming et al., 2016).

For many years, bioaccumulation in biofilms has been identified for a wide variety of contaminants, such as metals (Arini et al., 2012; Morin et al., 2008), pesticides (Headley et al., 1998; Tien et al., 2013), hormones, pharmaceuticals and endocrine disruptors (Huerta et al., 2016; Writer et al., 2011). More recently, various studies have used the properties of biofilm to accumulate substanc-

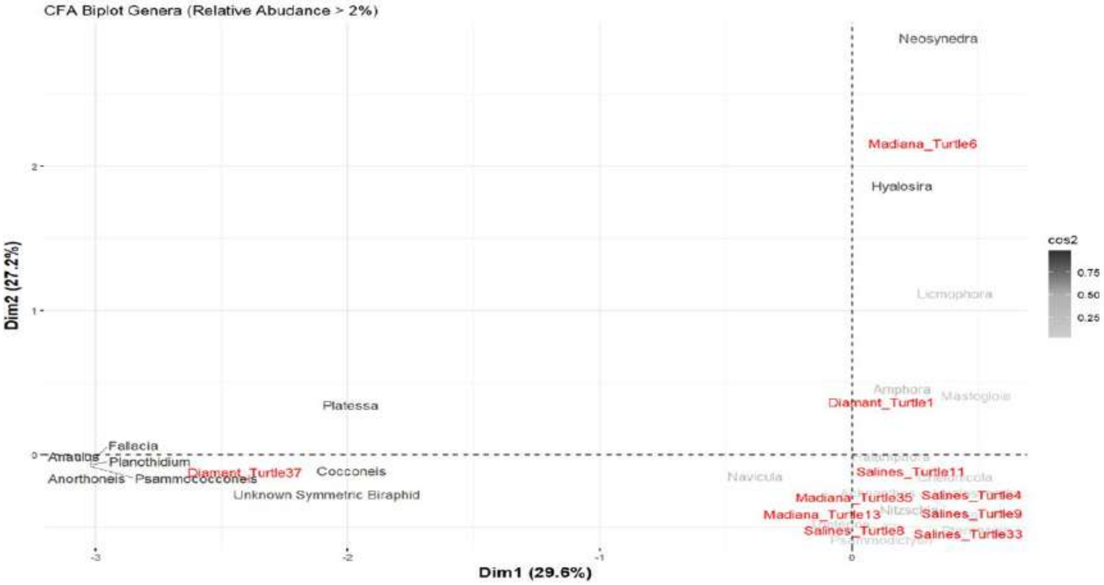


Figure 9. Correspondence Factor Analysis (CFA) based on genera (relative Abundance > 2%) and all samples (Beach/ Turtle). Row scale (genera) expressed in cos2 (relative contributions varying from 0 to 1).

es as a natural sampling tool. Hobbs et al. (2019) used biofilms to track the sources of PCB and metal contamination in rivers in the United States. Rooney et al. (2020) assessed the concentrations of 20 pesticides in periphyton in a coastal marsh and showed that more pesticides were detected in periphyton than in sediment. Likewise, Mahler et al. (2020) proved that stream biofilm sampling is an effective complement to sediment sampling, facilitating the identification of additional pesticides. Zhang et al. (2022) demonstrated that biofilm can act as a potential integrative sampler, in which PFAS concentrations (per- and polyfluoroalkyl substances, synthetic organofluorine compounds) are more representative than point water samples. Among contaminants in aquatic environments, plastic microparticles are of growing concern due to their large quantity, wide distribution and adverse effects. The presence of plastic microparticles provides a new artificial substrate for biofilms. Several studies have shown that these microparticles are colonized by different biota, in particular by diatoms and bacteria (e.g. Barnes, 2002; Oberbeckmann et al., 2015; Parrish & Fahrenfeld, 2019; Reisser et al., 2014; Sooriyakumar et al., 2022; Zettler et al., 2013). Most studies have focused on microplastic particles in surface waters. Although many plastics float, microplastics are exported to sediments after colonization (Kooi et al., 2017; Long et al., 2015). A few studies have addressed the issue of biofilm formation on plastic in marine sediments (Harrison et al., 2014; Pinnell and Turner, 2019). Moreover Seeley et al. (2020) found that the presence of plastic microparticles alters the structure and function (nitrogen cycle processes) of microbial communities in coastal salt marsh sediments. Recently Pertiwi et al., (2022) have shown that, in the Lahor Reservoir (Indonesia), the abundance of plastic microparticles in biofilm is greater than that of water and that biofilm can therefore be used to monitor water pollution by microplastics. Likewise Huang et al. (2021) found that microplastics were concentrated in the biofilms of a canal. According to Kalčíková & Bundschuh, (2021), the retention of microplastics by natural biofilms (temporary or long-term) has not yet been studied, but some laboratory studies suggest that it may be important. But to our knowledge, no study has looked for the presence of these microparticles in epizoic biofilms. However, it is very likely, given the distribution of these microplastics, that they are also present in the benthic communities of turtle shells. Thus, if contaminants (chemical substances or plastic micro-particles) accumulate on, and within this matrix, biofilms are a potentially very rich source of information for studying the contamination of sites frequented by turtles.

The adsorption capabilities of the EPS matrix are not limited to contaminants in the environment. Two recent studies suggest that aquatic stream biofilms can physically act as environmental sensors of environmental DNA (eDNA) (Rivera et al., 2022, 2021). In their first study, Rivera et al. (2021) compared morphological inventories of macro-invertebrates and molecular inventories based on macro-invertebrate eDNA extracted from biofilms in rivers in Mayotte. The results showed that the two methods provided comparable structures and diversities for macroinvertebrate communities (when using molecular inventories in OTU and not after taxonomic assignment) suggesting that macroinvertebrate eDNA present in biofilms is representative of the station community. In their second study, (Rivera et al., 2022) collected biofilms and water samples from the coastal sites of a large European lake (Lake Geneva). Molecular inventories from biofilm and water samples provided comparable taxonomic lists. These results open up prospects for studies on the eDNA present in biofilms. Thus, from the same matrix, the

Table 3. The number of diatoms taxa in the most diverse genera in samples from different beaches. A = Presence and B = Counting.

Genera	Overall	Counting	Diamant		Madiana		Salines	
			A	B	A	B	A	B
Nitzschia	173	86	42	20	117	148	87	94
Navicula	168	64	46	27	102	56	70	26
Cocconeis	91	34	32	21	59	28	50	24
Halamphora	77	47	24	21	43	48	37	31
Amphora	66	24	23	9	43	37	22	23
Mastogloia	63	17	28	3	40	11	28	15
Licmophora	41	16	15	2	21	11	20	6
Diploneis	36	6	10	4	22	5	11	1
Fallacia	33	15	11	19	26	17	12	8
Seminavis	30	14	8	0	16	12	14	8
Planothidium	28	13	7	10	21	13	11	7
Achnanthes	26	16	6	4	14	12	17	28
Hyalosira	23	8	11	4	13	3	4	3
Achnanthidium	17	6	4	3	6	2	10	3
Cocconeiopsis	15	7	6	5	6	10	9	3
Caloneis	14	2	4	0	11	2	3	1
Opephora	14	5	3	0	9	4	4	1
Thalassiosira	14	4	3	0	14	8	1	0
Platessa	12	8	4	10	4	15	10	12
Psammodictyon	12	7	4	4	10	11	6	7
Rhopalodia	12	4	3	0	9	2	4	3
Grammatophora	11	5	3	0	5	0	8	5
Hyalosynedra	11	4	6	5	5	9	5	12
Tabularia	11	0	1	0	7	0	4	0
Parlibellus	10	4	4	3	7	2	3	0
Olifantiella	9	5	2	0	7	8	1	1
Others	434	134	146	90	278	122	133	72
TOTAL	**1451**	**555**	**456**	**264**	**915**	**596**	**584**	**394**

biofilm of turtle shells, it would be possible to extract DNA and study the community of diatoms, fungi and bacteria constituting the biofilm, but also the eDNA of macro-invertebrates, fish and marine mammals captured by the biofilm. If the quantity of eDNA collected is sufficient, this global study would give indications on the organisms that share the turtles' environment and thus provide valuable information on the routes of dispersal.

The work to standardized protocols to allow interspecific, biogeographic and environmental comparison have to be continued. Genera and species richness of diatom communities from turtle shells are largely underestimated. More investigations are required to determine how many taxonomic units should be counted to approximate true specific diversity of diatom communities of turtles.

Turtle diatom communities are not correlated with the nesting beaches, but differ significantly. The observed differences could be correlated with the feeding environments. To complete our study, a priority is to get the environmental Martinique data and the diatom data for seagrass beds for comparative analyses. These data would allow the identification of the different feeding areas of the turtles.

It would be also necessary to expand the geographic area in which turtle samples are collected to other Caribbean Islands and other countries as turtles travel to all Caribbean islands and much further afield.

At last, further investigations should be carried out 1/ to get diatom data on other turtle species to interspecific comparison, 2/to perform an early sampling in the egg-laying season to allow “recapture” for intra-individual comparison and 3/ to have a multi-year monitoring for year-to-year comparison.

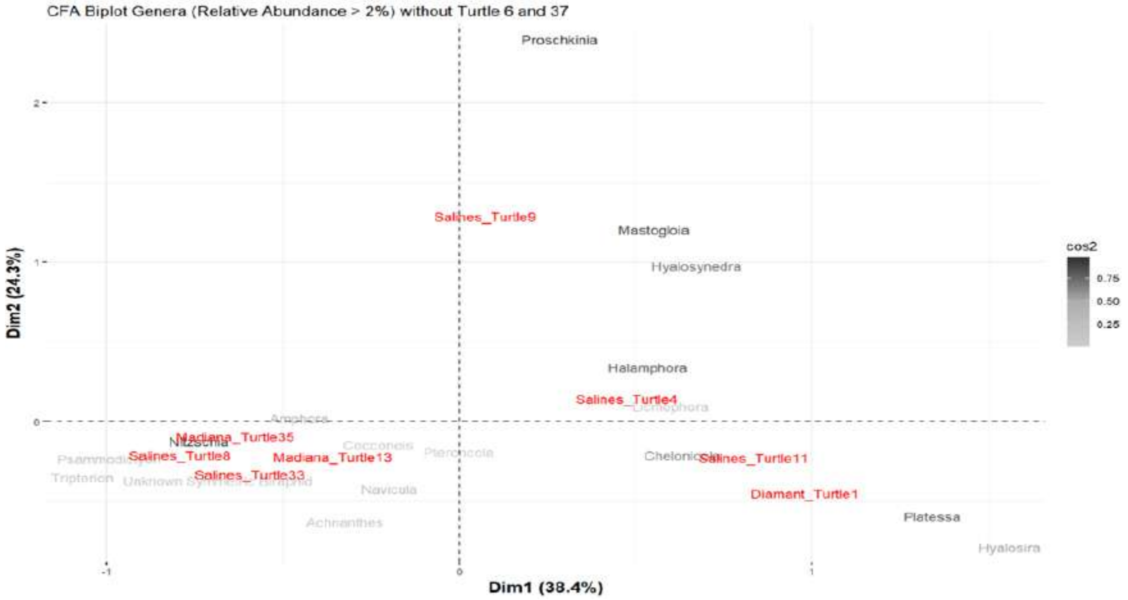


Figure 10. Correspondence Factor Analysis (CFA) based on genera (relative Abundance > 2%) and samples (Beach/ Turtle) without Turtles 6 and 37. Row scale (genera) expressed in cos2 (relative contributions varying from 0 to 1).

The other way of studying turtle diatoms is DNA metabarcoding for diatom identification to improve data on turtle diatom communities.

The experimental way of using environmental DNA data of biofilm and the adsorption capacity of EPS needs to be tested. These methods make it possible to learn about other organisms living with turtles and about environmental contaminations of turtle habitats.

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Factors of distribution

Among the studied factors that may explain the regional variations in spotted eagle ray sightings, fishing pressure and the type of fishing gear used appear to play a significant role. Our study revealed a notable decrease in sightings in areas where gillnets are used (IRR = 0.50, p-value = 0.005). Due to its swimming and feeding behavior, the spotted eagle ray is particularly vulnerable to coastal fishing gear, including gillnets (trammel nets and straight nets). This species exhibits a benthopelagic lifestyle, moving above sandy bottoms and seagrass beds while foraging, and swimming in open water when migrating. These behaviors make it especially susceptible to gillnets, which are often placed along coastal habitats such as sand flats, seagrass meadows, and reefs (Hoenig et al. 1990 ; Schluessel et al. 2010).

Gillnet fishing is widely practiced in the FWI, particularly in Guadeloupe and Martinique, where the demand for coastal fish remains high. In contrast, in Saint-Barthélemy and Saint-Martin, consumer preferences lean towards pelagic fish, resulting in lower gillnet usage in coastal areas (com. pers. Beaufort O.). In 2023, nearly 155 vessels in Martinique and 200 in Guadeloupe were reported to use gillnets (IFREMER, 2024a et b), highlighting the significant fishing pressure in these territories. Conversely, gillnet fishing is highly regulated in Saint-Barthélemy, where only the “folle à lambis”, a traditional net used for queen conch fishing is permitted. This practice is limited to a specific period each year, with less than 10 fishers using it (com. pers. Muller E.). These differences in fishing intensity likely contribute to the higher ray sightings in Saint-Barthélemy compared to the other territories.

Differences in species-specific conservation measures further contribute to the observed regional variations in spotted eagle ray populations. In Saint-Barthélemy, a fishing ban was implemented in 2015, and the species was officially classified as protected in 2020. However, in Guadeloupe, spotted eagle rays continue to be actively fished and are frequently observed at local fish markets. In Martinique, protective measures were introduced in August 2019 through prefectural decrees, which included a ban on fishing the species as well as a prohibition on the use of trammel net (a type of gillnet known to have a significant impact on elasmobranch populations in the FWI) (com. pers. Beaufort O.). While these regulatory

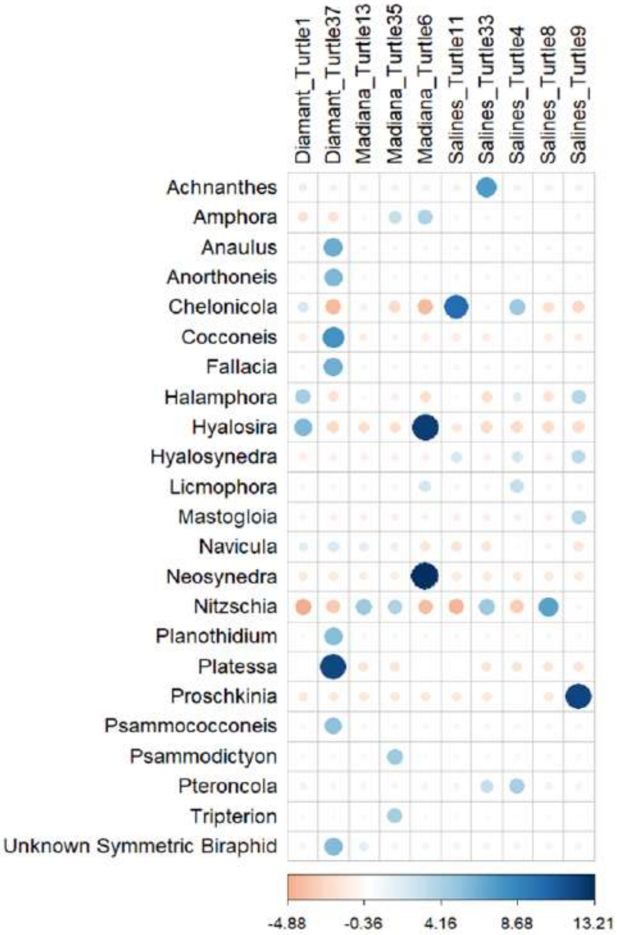

Figure 11. Corrplot of Pearson's residual value for genera (Pearson's Chi-squared test, p-value < 2.2e-16). For a given cell, the size of the circle is proportional to the amount of the cell contribution. Genera with relative abundance > 2%.

actions represent a critical step forward for the conservation of spotted eagle rays in Martinique, the low encounter rate recorded in the region suggests that local populations remain in a highly concerning state despite these regulatory measures. It is possible that conservation efforts were iniciated too late, after populations had already been severely depleted. Furthermore, the long recovery times associated with the species' low resilience, characterized by population doubling times exceeding 14 years (according to FishBase), suggest that the positive effects of these measures may take substantial time to materialize.

Other human activity-related factors may also contribute to the observed differences in ray populations across the FWI. One key factor is the variation in Marine Protected Area (MPA) strategies between territories, including differences in size, number, distribution and regulations. In Saint-Barthélemy, which has approximately 40 km of coastline, including its islets, there are five coastal natural marine reserves covering 1200 hectares, distributed across different parts of the island. Although regulations vary between these reserves, elasmobranch fishing is strictly prohibited, and human activities are regulated to varying degrees across the zones. Similarly, in Saint-Martin, which also has about 40 km of coastline, the marine area of the nature reserve spans 2900 hectares and includes the majority of the island's coral reef formations. In contrast, Guadeloupe, with approximately 650 km of coastline, has three MPAs: two marine parks and one marine nature reserve, covering a total of 4152 hectares. For the marine park, only the designated 'core' zone, with strict rules governing human activities, was considered in this study. Martinique, with nearly 450 km of coastline, has a single marine nature reserve covering 603 hectares. While a marine park designation exists across all territorial water, this classification does not necessarily indicate a fully protected MPA with strict regulations but rather represents a governance framework for marine management. A comparison of the ratio of strictly protected marine area to coastline length, highlights notable differences between territories. Saint-Martin and Saint-Barthélemy have relatively high ratios (72.5 hectares/km and 30 hectares/km, respectively), compared to Guadeloupe and Martinique (0.68 hectares/km and 0.15 hectares/km, respectively). These disparities likely influence the conservation of marine species, and therefore may contribute to the observed variation of spotted eagle rays in the FWI. The distribution and the size of MPAs in the FWI may be a key factor in their effectiveness for mobile species like the spotted eagle ray. Genetic and seasonal variation researches confirm the presence of migration patterns within the Caribbean and Gulf of Mexico (Bassos-Hull et al., 2014; Sellas et al., 2015), with mouvements of up to 20 km within the same territory (Flower, 2017) which may limit the protective effect of small, localized MPAs in some territories. This could partially explain why sightings are more frequent in the Grand Cul-de-Sac Marin (GCSM) and Nord Grande-Terre (NGT) sectors in Guadeloupe, which are located within or adjacent to the core protection zone of the marine park.

Another significant factor influencing ray sightings is seasonality. Our results indicate that spotted eagle ray encounters increase significantly toward the end of the year, with the highest sightings occurring between October and December (IRR = 1.58, p-value < 0.001). Similar seasonal fluctuations have been observed elsewhere in the Caribbean and the Gulf of Mexico, suggesting that these fluctuations are driven by seasonal movements. For instance, in Sarasota Bay, Florida, where sea temperatures can drop by almost 10 to 15°C in winter, studies have recorded a decline in abundance during the coldest months (Bassos-Hull et al., 2014; Sellas et al., 2015). Conversely, in southeastern Mexico, Cuba, and Venezuela, where water temperatures remain relatively high year-round, spotted eagle ray numbers peak in winter (Cuevas-Zimbrón et al., 2011; Tagliafico et al., 2012; Ruiz A., unpublished data). Thermal preference is a well-

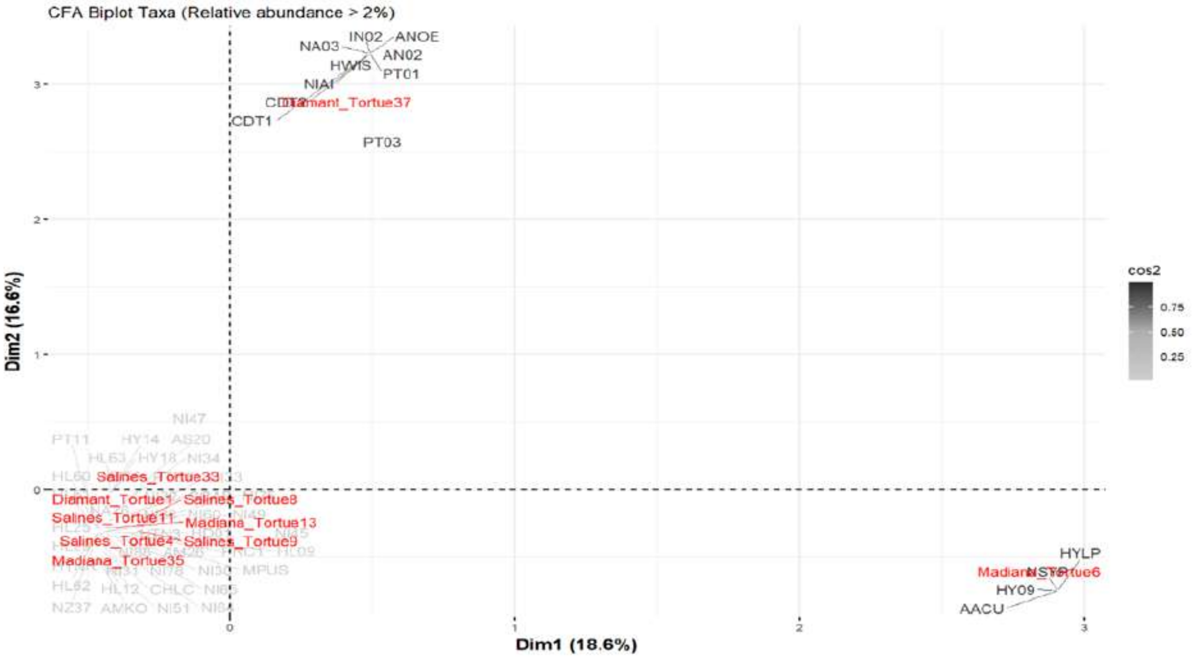

Figure 12. Correspondence Factor Analysis (CFA) based on taxa (relative Abundance > 2%) and all samples (Beach/Turtle). Row scale (taxa) expressed in cos2 (relative contributions varying from 0 to 1). Taxa and correspondence between taxa code and species name listed supplement Table 2.

documented factor influencing movement patterns in marine species, as organisms select optimal temperatures to maximize physiological performance, including growth rates and reproductive success (Heupel et al., 2007; Garla et al., 2006). These findings suggest that seasonal migrations linked to temperature fluctuations may explain the observed patterns in spotted eagle ray sightings in the FWI. However, further research is needed, particularly through satellite tracking and genetic studies, to better understand regional movement patterns and potential connectivity between populations.Regarding the moon, although lunar cycles are known to influence marine animal behavior, our study found no significant relationship between the lunar phase and spotted eagle ray sightings (IRR = 1; p-value = 0.7). This result contrasts with previous findings, such as those of Cuevas-Zimbrón et al. (2011), who reported increased ray captures in fisheries during the new moon in the southern Gulf of Mexico. The lack of a lunar effect in our data suggests that spotted eagle ray movements in the FWI may be influenced more by seasonal factors than by lunar cycles. However, further investigations are needed to clarify the potential interactions between lunar phases and ray populations across different regions.

While the region of the FWI is characterized by a tropical climate, significant environmental variations exist across the different territories. In some areas, deep waters are located close to the shoreline. Whereas others feature extensive shallow water of less than 25 meters in depth, such as the Grand Cul-de-Sac Marin in Guadeloupe, which is protected by a largest barrier reef. Additionally, anthropogenic activites vary considerably across these sectors, particularly in terms of fishing practices and tourism. These environmental and socio-economic differences highlight the need for conservation strategies tailored to the specific conditions of each territory.

Method and model improvement

As with any monitoring method, our citizen science program has certain limitations that must be acknowledged. Specifically, these limitations are often related to : i) species-level identification, ii) counting individuals, iii) consistency in protocol application, and iv) participant availability. However, in the context of this study, the limitations associated with the first two points are less significant due to the nature of the spotted eagle ray. This species is relatively easily identifiable and is known for generally living in solitary habits, though it can occasionally form small schools of up to a dozen individuals (*Bassos-Hull, 2014*), making species identification and individual counts more straightforward.

To ensure feasibility within diving clubs, the protocol was intentionally designed to be simple and easily integrated into regular diving activities. While this simplicity facilitates long-term engagement from dive instructors, it also limits the ability to gather environmental data on key variables that could influence ray populations. Therefore, while the protocol was essential to encourage participation, it also restricted the depth of

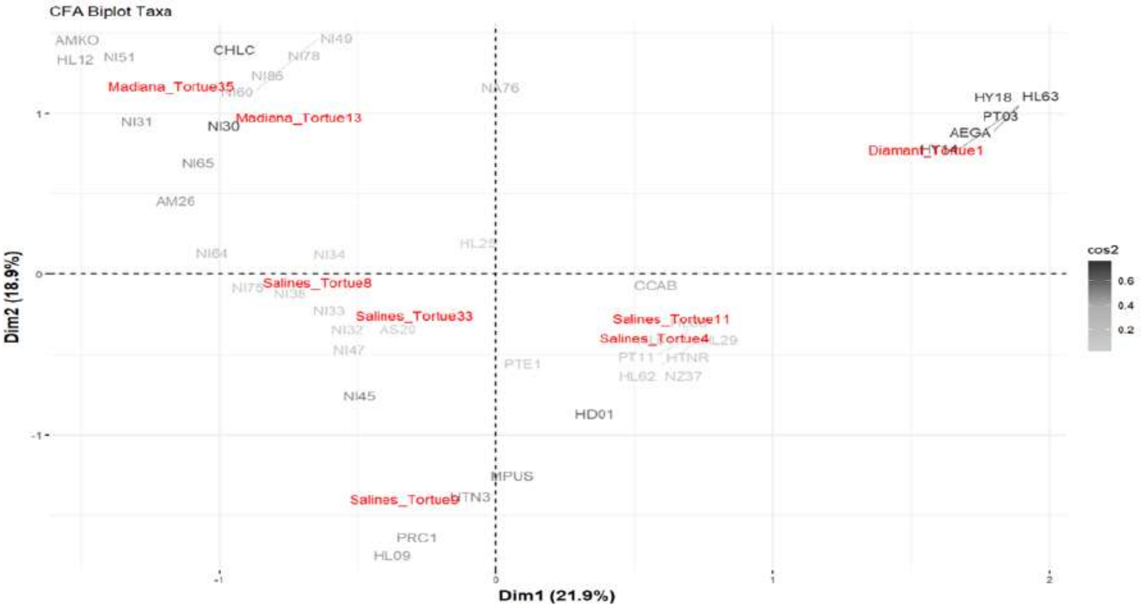

Figure 13. Correspondence Factor Analysis (CFA) based on taxa (relative Abundance > 2%) and samples (Beach/Turtle) without Turtles 6 and 37. Row scale (taxa) expressed in cos2 (relative contributions varying from 0 to 1). Taxa and correspondence between taxa code and species name listed supplement Table 2.

additional ecological data that could further enhance the understanding of spotted eagle ray distribution. This study was based on the available data at the time of analysis, highlighting the need for continuous data collection on additional factors that were not initially considered such as temperature and depth for each sighting. Acquiring supplementary data on variables not included in this study could improve the model and provide a deeper understanding of spotted eagle ray populations in the FWIAccording to the literature, factors such as physico-chemical parameters (e.g., temperature, current, and tide; *Berthe, 2016; Cuevas-Zimbrón et al., 2011*), biotic factors (e.g., predation pressure, prey availability, and the presence of conspecifics; *Collins et al., 2007; Heithaus et al., 2009; Jaine et al., 2012; Ajemian, 2012*), ecological factors (e.g., habitat type and condition), and anthropogenic factors (e.g., maritime traffic; *Berthe, 2016*) can all directly influence ray populations and sightings. Additionally, factors that directly impact the likelihood of sightings, such as turbidity, dive type (e.g., training dive, exploration dive, etc.), dive duration, and observer bias, should be taken into account. The latter, in particular, can be addressed by involving multiple instructors from the same diving club to participate in the program on the same dives, which would allow for replicates and reduce the potential for individual bias. Future studies would benefit from the inclusion of these variables, enabling more comprehensive monitoring that could lead to a better understanding of the spatial distribution and population dynamics of spotted eagle rays in the FWI.

CONCLUSION

Citizen science programs, though often undervalued in comparison to traditional scientific initiatives, play a crucial role in enhancing our understanding of marine species and ecosystems. This study demonstrates that without the engagement of citizen science, we would not have been able to gather the extensive data necessary to assess the status of spotted eagle ray populations across the FWI. The broad sampling effort provided by local dive clubs has significantly advanced our knowledge of this coastal species, currently threatened with extinction and in urgent need of conservation intervention. While this approach comes with inherent limitations, the study also emphasizes the potential for adaptations that can mitigate these challenges and improve the quality of future data collection.The marked differences in spotted eagle ray sightings across the FWI reflect varying population statuses across territories.

This study reaffirms the role of Saint-Barthélemy as a "hot spot" for coastal elasmobranchs in the region (*Beaufort, 2023*) while emphasizing the need for urgent conservation efforts in Guadeloupe and Martinique. These regions are facing mounting pressures on ray populations, making it imperative to mitigate threats, from fishing activities and coastal habitat degradation. The spotted eagle ray plays a dual ecological role as both predator and prey, playing a critical part in maintaining the the balance and health of coastal ecosystems (*Bernett et al., 2010, Serrano-Flores et al., 2018*). Beyond its ecological importance, this species holds significant ecotouristic value. As a charismatic marine species, it can attract divers and snorkelers, thus offering economic opportunities for local communities (*Flowers, 2017*). Tourism

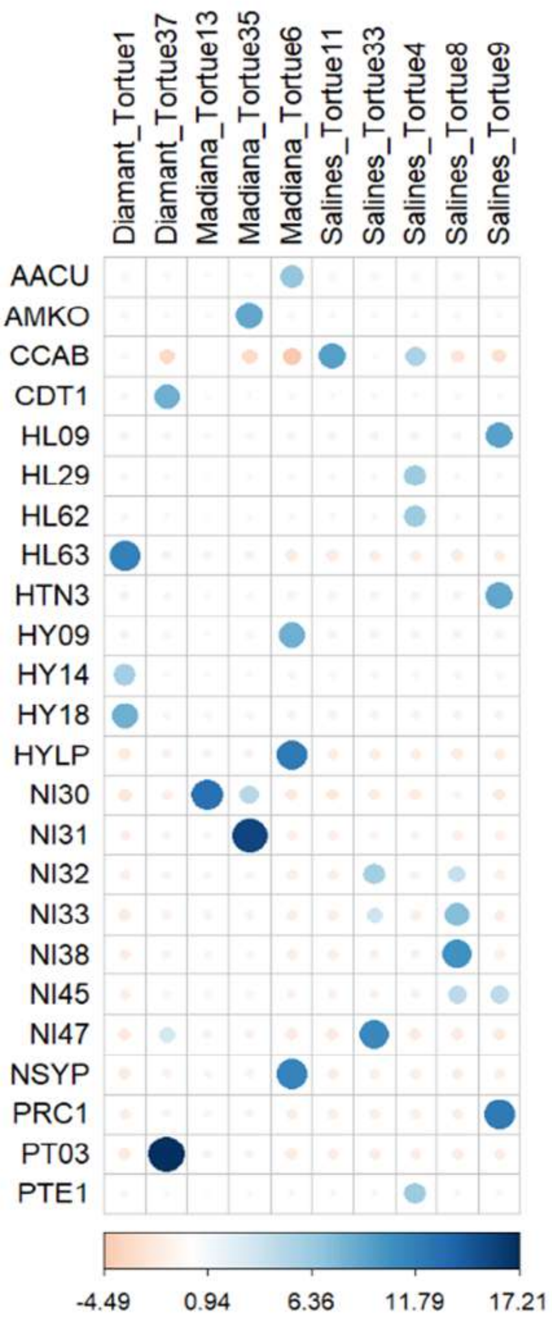

Figure 14. Corrplot of Pearson's residual value for species (Pearson's Chi-squared test, p-value < 2.2e-16). For a given cell, the size of the circle is proportional to the amount of the cell contribution. Species with relative abundance > 5%. Taxa and correspondence between taxa code and species name listed supplement Table 2. square.

operators in the FWI are already recognizing this potential, with some promoting spotted eagle ray sightings as a key attraction (pers. comm.). When effectively managed, elasmobranch-focused ecotourism has the potential to generate millions of dollars annually, benefiting national economies while simultaneously promoting conservation efforts (*Anderson et al., 2001; Gallagher et al., 2011; Haas et al., 2017*). This approach aligns with the growing trend of marine-based tourism that supports both local livelihoods and environmental preservation.Among the factors influencing ray populations, some are easier to address than others. While natural variables like seasonality are beyond human control, fishing pressure can be significantly reduced through effective management strategies. Regulations concerning the spotted eagle ray vary across the FWI territories, with Saint-Barthélemy offering the highest level of protection, where fishing was banned since 2015 and the species was classified as protected in 2020. In Martinique, a fishing ban was implementedin August 2019, while Guadeloupe and Saint Martin have prohibited recreational fishing for the species. However, enforcement of these regulations presents challenges, as certain fishing gear, such as the "folle à lambis" (a type of gillnet), is still allowed in some areas, including Martinique and Saint-Barthélemy. This particular net is particularly problematic as it inadvertently captures spotted eagle rays, especially given its similarities to the "folle à raie", a net specifically designed for rays (pers. comm. from fishermen).

Despite these regulations, the low observation rate of spotted eagle rays in Martinique, where both the species' fishing ban and the prohibition of trammel nets have been in place since 2019, underscores the urgency of conservation action. This suggests that existing measures, while important, have not yet succeeded in reversing population threat. The spotted eagle ray's high vulnerability to fishing pressure, combined with its limited resilience, makes it particularly susceptible to population declines. Given this, it is critical to act swiftly to mitigate the threats we can influence, particularly fishing pressure and the degradation of coastal habitats. Beyond enforcing existing protections, it is crucial to combat habitat loss and mitigate other anthropogenic pressures to ensure the long-term survival of this ecologically and economically valuable species

In addition to these challenges, the distinct administrative and regulatory frameworks of each FWI territory add complexity and significantly influence environmental governance and conservation policies. These challenges underscore the need for targeted, localized conservation efforts tailored to the specific fishing practices and threats faced by each territory. Additionally, it is crucial to harmonize monitoring and conservation measures not only across the FWI but also throughout the species' entire range to ensure a cohesive and effective approach to its protection. Effective conservation will also require collaboration with local stakeholders, particularly fishermen, whose insights into fishing practices and knowledge of potential capture reduction techniques are invaluable. Consultation, knowledge exchange, and awareness-raising initiatives will be key to ensuring that conservation measures are both practical and effective in mitigating the impact on ray populations across the FWI.

Only through such concerted efforts can we work toward securing the long-term survival of the spotted eagle ray and other elasmobranch species in the region.

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KEYWORDS: Hawksbill sea turtle, Commercial Diatom, West Indies Island, exploratory analysis, taxonomic diversity.

SUPPLEMENTAL MATERIALS

Available from the authors:
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- **S1 TABLE.** *List of taxonomic publications used for identification of diatom taxa on Hawksbill see turtles.*
- **S2 TABLE.** List of 1451 taxa observed on Hawksbill turtle shells.